Coping with climate change: exploration of metabolic acclimation across generations in a tropical fish.

Honors Research Thesis

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Abstract

Human-induced climate change is altering the environment, presenting survival challenges to many organisms. In aquatic environments, rising water temperature is among the most prevalent manifestations of climate change stress. When faced with water warming, one way for populations to persist is for its individuals to acclimate to the new temperature. However, the potential for acclimation may be limited, especially in tropical species that likely evolved in a narrow range of environmental conditions. Herein, we seek to determine the capacity for cross-generational acclimation in *Julidochromis ornatus*, a tropical cichlid species endemic to, but common in, Lake Tanganyika (East Africa). A previous experiment showed that adult (F_0 generation) *J. ornatus* individuals exposed to the anticipated Lake Tanganyika water temperature at year 2100 (29°C) experienced an increase in mass-specific metabolic rate, which never declined to baseline levels even after 6 mos of exposure and subsequent reductions in reproductive output and age-specific growth rate. By contrast, no change in mass-specific metabolic rate was observed in fish held at baseline (historically normal) temperature (i.e., 25°C). For our study, we quantified the mass-specific metabolic rates of the progeny of these F_0 adults (i.e., F_1generation individuals), which spent their entire lives in either 25˚C or 29˚C water. In so doing, we tested the hypothesis that the F_1generation individuals would have a lower mass-specific metabolic rate at 29°C than F_0-generation individuals, owing to maternal (epigenetic) effects. Herein, we present findings regarding the ability of the second (F_1) generation individuals to alter their mass-specific metabolic rate and then discuss what these results might mean for population persistence in this and other tropical cichlid species that are experiencing climate-driven water warming.

Introduction

Human activity is rapidly altering the environment and creating unique challenges for organisms all over the world (Sih et al. 2011). In aquatic ecosystems, such challenges include habitat loss and habitat shifts that are outside of an organism's physiological tolerance, most notably temperature increases (Portner and Knust 2007; Morrongiello et al. 2011). In the face of such challenges, individuals can move to more tolerable habitat, acclimate to the new conditions (without experiencing genetic change), adapt to the new conditions (i.e., experience natural selection that can alter genotypes), or face extinction (Hamilton and Miller 2015). Countless species are thought to be facing extinction and much uncertainty exists as to whether they will be able to respond in a way that ensures their continuity (Morrongiello et al. 2011; Garcia-Robledo et al. 2015).

Ectotherms, such as fish, are particularly vulnerable to increased environmental temperatures that can lead to increased metabolic rate and subsequent negative effects on reproduction and growth (Portner and Knust 2007; Donelson 2011). The capacity for and extent to which fish can acclimate to climate-driven increases in water temperature can be assessed by measuring metabolic rates before, during, and after exposure to elevated temperatures (Donelson et al. 2011). An ideal investigation, would measure metabolic rates across generations, as exposure to an environmental stress in one generation could allow for future generations to better acclimate or cope through epigenetic effects (Munday 2014).

Herein, we sought to determine if transgenerational acclimation in metabolic rate is possible in the tropical freshwater fish, *Julidochromis ornatus* (Golden Julie), which is an abundant species that is only found in Lake Tanganyika (east Africa). This cichlid species is an ideal study organism because Lake Tanganyika has been warming at an unprecedented rate, owing to human-induced increases in

greenhouse gas emissions to the atmosphere (Tierney et al 2010). *J. ornatus* also is distributed throughout the lake and is ecologically representative of the other endemic cichlids in the lake (Awata et al 2005). Our study builds upon a previous study (Brodnik 2015) that was conducted with the parental generation of the study individuals used in this investigation. Brodnik (2015) exposed *J. ornatus* adults of the same age and breeding population—none of which had prior exposure to high temperature during the egg, larval, or juvenile stage—to both current (baseline; 25°C) and projected future (29°C) Lake Tanganyika water temperatures in a controlled laboratory experiment. He found that the individuals exposed to the higher (projected future) temperature experienced increased mass-specific metabolic rates and reduced fitness (e.g., reduced age-specific growth rate and reproductive output) relative to individuals exposed to the lower (baseline) water temperature. Given that the mass-specific metabolic rates of the parental adults never dropped to the levels observed in the low-temperature treatment, even after 6 mos of study, Brodnik (2015) concluded that this species was unable to acclimate to increased water temperature, and might face extinction risk in the absence of acclimation on the part of future generations or rapid evolution.

By quantifying the mass-specific metabolic rate in the F_1 offspring of the fish used in Brodnik's (2015) investigation (from both temperature treatments), we sought to determine if transgenerational acclimation to elevated temperatures is possible in this species. Evidence of this phenomenon would be the second generation (F_1) individuals in the high-temperature treatment—which spent their entire lives (from prezygotic stages to adults) at an elevated temperature—showing an average metabolic rate that is lower than in the previous (F_0) generation, and hence, more akin to the average metabolic rate of individuals in the low-temperature treatment. Such acclimation ability would be similar to what Donelson et al. (2011) showed in a tropical coral reef fish, and would point to the possibility that epigenetic change (e.g., maternal or paternal effects) can allow *J. ornatus* to cope with climate warming through changes in gene expression. If *J. ornatus* does have the capacity for transgenerational

acclimation, a more positive outlook than that proposed by Brodnik (2015) could be held, not only for *J. ornatus*, but also the endemic cichlid assemblages of the entire African Rift Valley region. Given the findings of Donelson et al. (2011), we hypothesized that exposure of second-generation (F_1) individuals to high temperature during their entire life-history would allow *J. ornatus* to demonstrate transgenerational metabolic acclimation, as evidenced by a reduced average mass-specific metabolic rate relative to the parental generation.

Methods

Study Species

The fish used in this experiment were the immediate offspring of the fish used in Brodnik's (2015) study, and hence, resided in the same experimental setup as the parental (F_0) generation. Both generations of fish were housed in 57 L tanks, with the F_0 generation residing in tanks as male-female breeding pairs (n = 2 fish per tank). By contrast, the F_1 individuals were housed in larger groups within a tank (n = 5-15 fish), as they had not yet formed breeding pairs. All F_1 offspring were maintained at the same water temperature as their parents (either 25°C or 29°C). Water temperature, pH, and dissolved oxygen were monitored daily, with water changes occurring at least every other week. Study organisms were fed two cichlid feed pellets (Hikari Sinking Cichlid Gold Pellets), similar to the F_0 generation, which provided sufficient food to meet their basal metabolic needs (Brodnik 2015). All procedures followed guidelines set forth in IACUC protocol #2012A00000112-R1 under PI Stuart Ludsin.

Mass-specific metabolic rate

Mass-specific metabolic rate was compared between 13 males in the parental (F_1) generation and 14 individuals in the second generation (7 males and 7 females). The fish in the two groups differed in mass, with the second generation fish being both smaller (mean F_1 = 4.24 ± 1.21 g; mean F_2 = 2.80 ± 0.55; $t(16) = 3.95$; $p = 0.00057$) and younger (F_1 = ~2.5 years old; F_1 = < 1 year old).

Dissolved oxygen consumption was used as a proxy for metabolic rate. Dissolved oxygen consumption was measured using the same intermittent-flow respirometry equipment and protocol as the previous experiment done on the F_1 generation, to allow for comparison across generations (Boisclair and Sirois 1993; Brodnik 2015). The respirometer consisted of a cylindrical class chamber (0.15 L), where the fish were placed, which was connected to a recirculating pump and in-line dissolved oxygen probe (manufacturer). The probe measured and recorded dissolved oxygen 10 times/min. Current in the system was restricted to the lowest level possible such that fish were could maintain position without needing to swim. In so doing, we sought to approximate the basal metabolic rate as best as possible. However, maintaining water circulation was important to ensure homogeneous dissolved oxygen concentrations (Boisclair and Sirois 1993). The system was connected to a tank of oxygenated water of identical temperature (either 25°C or 29°C) that was used to replenish the dissolved oxygen of the respirometry system via gravity in between cycles of each trial.

Fish were deprived of food for 24 hours prior to metabolic rate testing to eliminate effects of food metabolism on measured oxygen consumption (Sandblom et al. 2014). Each trial began with a blank run (i.e., 20 min without the fish in the chamber) to determine the background rate of oxygen depletion in the system. After the blank run, a fish was placed into the respirometry chamber and six cycles of intermittent-flow respirometry were conducted. One cycle lasted 30 min or until the dissolved oxygen level was 80% of the starting value. After one cycle, a valve was opened allowing oxygenated water to flow into the system for 3 min to replenish oxygen levels in the system before closing the valve to begin the next cycle. Immediately after concluding the 6^{th} cycle, the wet mass (nearest 0.1 g) of the

individual was measured volumetrically. The metabolic rate of each individual was determined by averaging the rate of dissolved oxygen depletion for only the last 3 (of the 6) cycles, and subtracting out the rate of oxygen consumption in the initial 30-min blank run (Brodnik 2015). Mass-specific metabolic rate was determined by dividing the average rate of dissolved oxygen consumption by the wet mass of the fish. Doing so allowed for comparison between fish of different masses.

Data analysis

We used a two-way ANOVA to test for differences among treatment groups. The factors included in our ANOVA model were temperature (25°C, 29°C), generation (F_0, F_1), and their interaction. An alpha level of 0.05 was used to denote significance. All data met assumptions of normality (Kolmogorov-Smirnov normality tests: all $P > 0.20$) and homogeneous variances (Levene's test: $P > 0.38$).

Results and Discussion

As expected, the F_1 generation had a lower mass-specific metabolic rate than the F_0 generation (mean F_0 = 0.22 ± 0.04 mg O_2/h/g; mean F_1 = 0.15 ± 0.05 mg O_2/h/g; $F_{1,25} = 14.4$, $P = 0.008$; Figure 1). Surprisingly, however, we did not find a strong temperature effect; no difference in mass-specific metabolic rate was observed as a result of temperature ($F_{1,25} = 0.19$, $P = 0.665$; Figure 2) nor was the generation X temperature interaction significant ($F_{1,25} = 0.041$, $P = 0.842$; Figure 3). Thus, while the metabolic rate of the F_1 generation was lower than the F_0, it declined in both temperature treatments.

Our results do not fully support our hypothesis that *J. ornatus* has the capacity for transgenerational metabolic rate acclimation in the face of rising water temperatures. We expected that individuals in the F_1 generation would exhibit reduced mass-specific metabolic rate relative to the

individuals in the F_0 generation, but only in the high temperature (29° C) treatment. In other words, we expected the mean mass-specific metabolic rate to be unchanged between generations in the low temperature group (25° C).

The unexpected reduction in mass-specific metabolic rate between generations for both temperature groups prevents us from drawing firm conclusions about the capacity for transgenerational acclimation to projected water temperature increases in *J. ornatus*. Although the results suggest the ability of this species to show adjustments to metabolic rate across generations, as to whether the observed change represents an innate capacity to acclimate to changing temperatures or a response to some other factor is unclear.

Our results differ from those of similar studies. Donelson et al. (2011) found that *Acanthochromis polyacanthus*, a tropical reef fish, was able to acclimate across generations, with fish exposed to elevated water temperatures at all stages of life displaying normal metabolic rates. Much like *J. ornatus* in Brodnik (2015), Donelson et al. (2011) found that *A. polyacanthus* experienced elevated metabolic rates when exposed to high temperature and that this elevated metabolic rate led to reduced growth and signs of lower reproductive output. Another study, which did not directly examine metabolic rate but rather growth rate, found that in *Cyprinodon variegatus,* a marine and estuarine minnow, showed transgenerational acclimation to elevated water temperature in the form of normal growth rates in subsequent generations (Salinas and Munch 2012).

Our inconclusive results could have been caused by several factors. First, the reduction in mas-specific metabolic rate seen in both temperature treatments may signify an artificiality of our experimental system. While we can only speculate, perhaps some domestication effect overshadowed the effects of temperature on metabolic rate. This explanation seems unlikely though as the study involving transgenerational acclimation in *A. polyacanthus* also housed fish in tanks in a highly

controlled laboratory setting much like our experiment and used a similar closed-style respirometry apparatus to measure metabolic rate (Donelson et al. 2011). Another hypothesis for our observed results relates to unanticipated artificial selection. Perhaps the only individuals that survived in any of the treatments were those that had the lowest metabolic rates. The possibility also exists that our study utilized too small of a sample size (n = 14) to detect a significant difference among treatments. To this end, while no significant differences were found between temperature treatments in the F1 offspring, the mass-specific metabolic rate was higher in the high-temperature treatment relative to the low-temperature treatment (see Figure 2). Lastly, it is conceivable that our results were influenced by the differences between the F_0 and the F_1 individuals. The F_0 group consisted of entirely males, whereas the F_1 group included both males and females. Additionally, the F_0 individuals were significantly larger and older than the F_0 offspring, and the F_0 generation were housed in individual breeding pairs, unlike the F_1 generation, which were maintained as a group (> 2 individuals per tank). For many species, it has been observed that metabolic rate can differ with age due to differing priorities for the energy of metabolism (e.g., growth vs. reproduction; Blueweiss et al. 1978). Further, like other African cichlids, *J. ornatus* can exist in cooperative breeding units that consist of more than two individuals (Awata at al. 2010). Thus, perhaps the larger group size may indirectly reduce metabolic rates for all individuals in some way.

Future Directions

Because we cannot know for certain to what extent mass, age, and group-size differences between the F_0 and F_1 individuals affected our results—and it is entirely possible that they had no effect—we encourage additional experiments, supplemented with field observations. Evidence of many marine species' ability to acclimate to the environmental effects of climate change is growing; however, a stark gap in knowledge still exists for tropical freshwater species (Foo and Byrne 2016; Sandlom et al.

2014; Donelson et al. 2011; Salinas and Munch 2012). McDonnell and Chapman (2015) found that *Psuedocrenilabrus multicolor*, another tropical African cichlid, had the ability to acclimate metabolically to variable temperatures but experienced a penalty to its resting metabolic rate when water temperature exceeded those of its natural range; however, research for many species has yet to address transgenerational acclimation to rising water temperatures. Understanding if *J. ornatus* or other tropical freshwater ectotherms, such as *Psuedocrenilabrus multicolor*, have the ability to exhibit transgenerational metabolic rate acclimation will be a crucial component to understanding how climate change will impact tropical ecosystems and may provide a less bleak forecast to the fate of many species (Foo and Byrne 2016).

Future work that examines *J. ornatus* or other species' ability to acclimate to unprecedented environmental conditions would benefit from looking at epigenetic changes in these organisms as a means of better understanding the mechanisms behind any observed acclimation. Epigenetic effects are heritable changes in DNA that result in altered gene expression but not a change in the DNA sequence, and such changes are thought to be the mechanism by which transgenerational acclimation is able to occur (Foo and Byrne 2016; Dupont et al. 2013). DNA methylation is the major way by which gene expression can be changed without modification of the actual DNA sequence, and one study found that *Crassostrea gigas,* an oyster, had widely varying methylation patterns among individuals exposed to varying environmental stresses (Gavery and Roberts 2010). Observing and characterizing epigenetic changes in *J. ornatus* that correlate to transgenerational acclimation to temperature changes could help illuminate how the organisms are changing in the face of environmental pressures.

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Figures

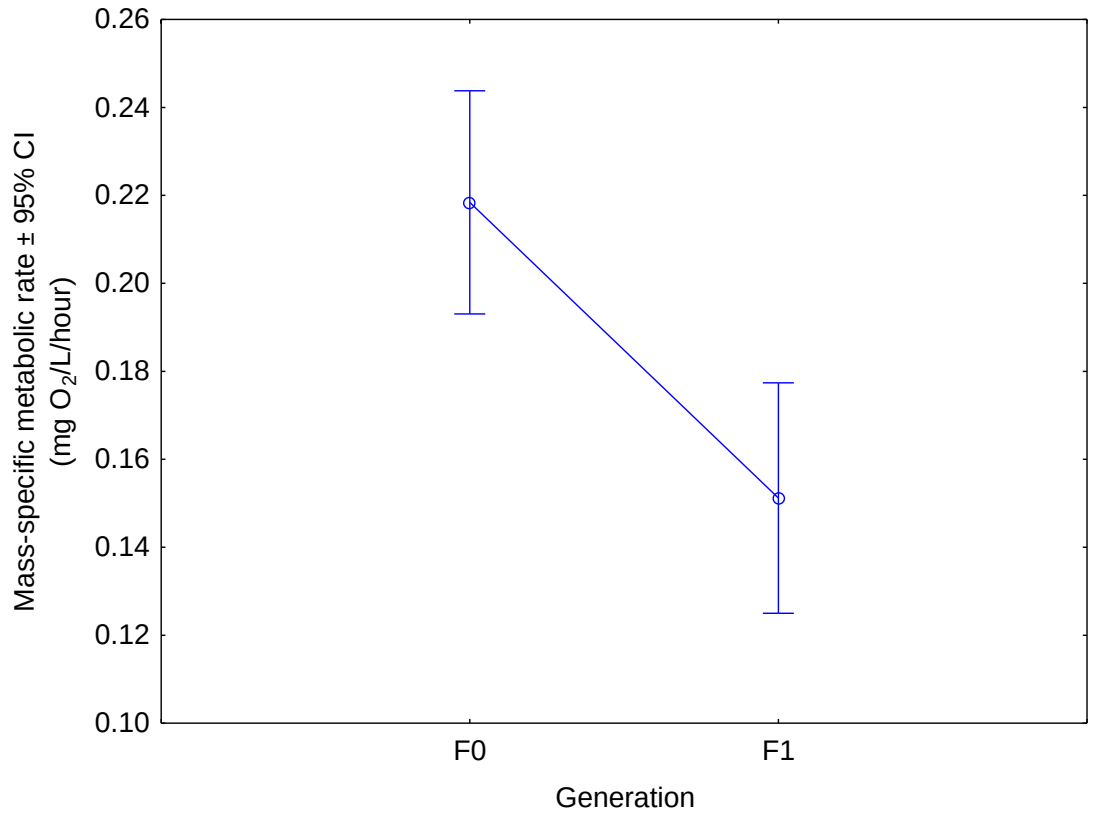

Figure 1: Comparison of second generation to first generation showing a significant decrease of mass-specific metabolic rate across generations. F(1,25)=14.418, p=0.000833; vertical bars denote 95% confidence intervals.

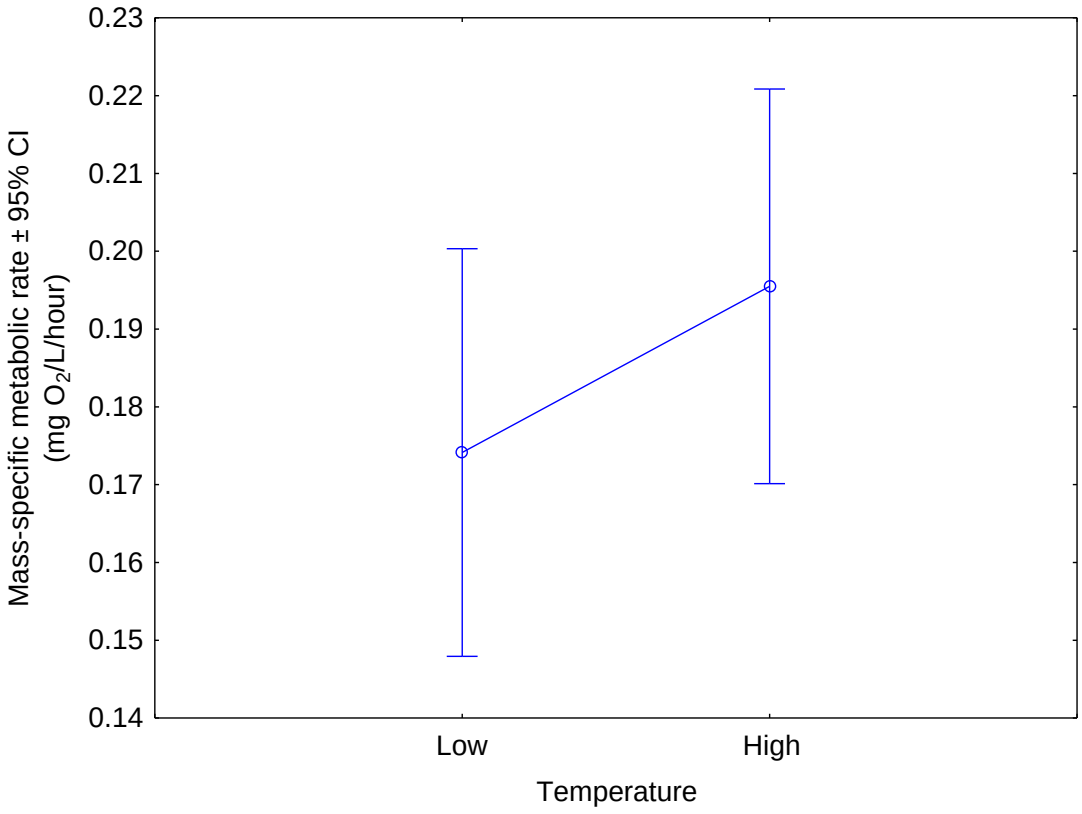

Figure 2: Comparison of high temperature (29°C) group to low temperature (25°C) group showing a slight but not significant difference in mass-specific metabolic rate due to the effect of temperature. $F(1,25) = 1.455$, $p=0.239$; vertical bars denote 95% confidence intervals.

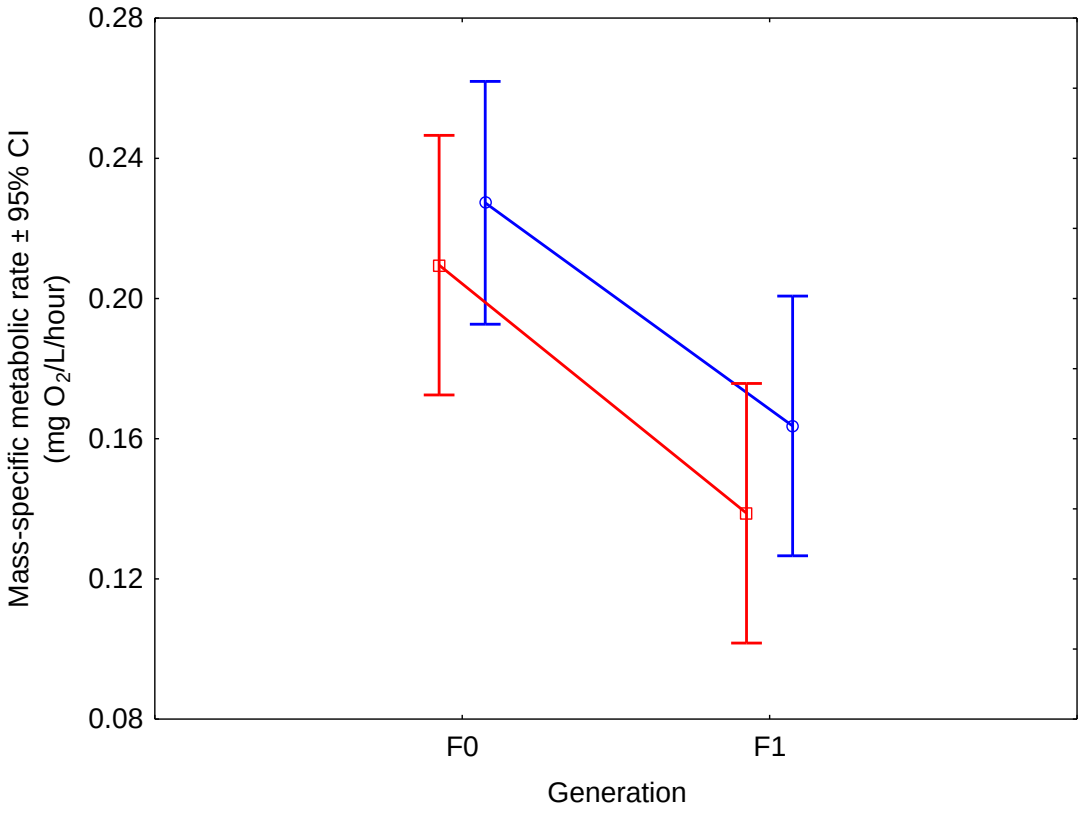

Figure 3: The interaction of both temperature and generation both failed to show a significant effect on mass-specific metabolic rate. $F(1,25)=0.0405$, $p=0.842$; vertical bars denote 95% confidence intervals.

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